Spatial Memory Performance 2 Weeks After General Anesthesia in Adult Rats

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We have previously demonstrated that general anesthesia with 1.2% isoflurane-70% nitrous oxide impairs acquisition of a radial arm maze task in both young and aged rats when testing begins 2 days after anesthesia and in aged rats when testing begins 2 wk later. We designed this study to examine whether postanesthesia learning impairment is persistent in young rats. Six-month-old rats were randomized to anesthesia for 2 h with 1.2% isoflurane-70% nitrous oxide, 1.8% isoflurane, or a control group that received 30% oxygen (n = 10 per group). Rats recovered for 2 wk and were then tested daily on a radial arm maze for 14 days. There were no differences between the controls and anesthesia groups in number of correct choices to first error or time to complete the maze. There was no main effect of group in terms of total number of errors (P > 0.05) but the group by day interaction was significant (P < 0.05), reflecting improved performance in the 1.2% isoflurane-70% nitrous oxide group relative to controls during the later days of testing (P < 0.005). Hence, in adult rats, previous general anesthesia is not associated with impaired learning 2 wk later. In fact, previous 1.2% isoflurane-70% nitrous oxide improves maze performance 2 wk later.

(Anesth Analg 2005;101:1389–92)

Clinical studies indicate that anesthesia and surgery are associated with early cognitive impairment in both young and aged patients (1,2). Recovery seems to be age-dependant, however, as only the aged suffer cognitive impairment lasting 3 mo or more (1,2). In the laboratory, we have demonstrated that a 2-h anesthetic with 1.2% isoflurane (ISO)-70% nitrous oxide (N₂O) attenuates performance improvement on a previously learned task in aged rats but improves performance in young adult rats (3). We found subsequently that acquisition of new spatial memory is impaired in both young and aged rats for 2–14 days after ISO + N₂O anesthesia and that this learning/memory impairment persists up to 28 days in aged rats (4,5). We have not determined whether postanesthetic learning impairment persists in adult rats but, given the greater plasticity of the young brain and the fact that postoperative cognitive impairment is short-lived in young and middle aged patients, we speculated that postanesthetic spatial memory impairment resolves sooner in adult rats. To test this hypothesis, we evaluated acquisition of spatial memory in adult rats beginning 2 wk after general anesthesia.

Methods

This study was approved by the Standing Committee on the Use of Animals in Research and Teaching, Harvard University Faculty of Arts and Sciences. Thirty 6-mo old male Fischer 344 rats were acquired from the National Institute of Health Aged rat colony at Harlan and housed individually in a climate-and humidity-controlled room on a 12-h light:dark cycle with continuous access to food and water. After a 1-wk acclimation period, rats were randomly assigned (n = 10 per group) to receive 1.2% ISO-70% N₂O-30% oxygen (ISO + N₂O), 1.8% ISO-30% oxygen (ISO), or 30% oxygen alone (control). Anesthesia was induced by placing rats in a chamber flushed with 3% ISO and 100% oxygen and intubating the trachea with a 14-gauge catheter. Rats were then mechanically ventilated with the appropriate anesthetic for 2 h with a 2-mL tidal volume delivered at a rate of 45 breaths/...
min, which pilot studies demonstrate maintains PaCO₂ at 41.4 ± 0.3 cm H₂O (mean ± SEM) in 6-mo-old male Fischer 344 rats. Rectal temperature was controlled to 37°C ± 0.5°C. Arterial oxygen saturation and mean arterial blood pressure (MAP) were measured noninvasively using a pulse oximeter and a tail cuff during anesthesia. After 2 h, the anesthetics were discontinued and 100% oxygen was delivered. The rate of ventilation was reduced until spontaneous ventilation resumed and the trachea was extubated when the rat was responsive. Control rats were placed in a box flushed with 30% oxygen for 2 h and were not tracheally intubated. All animals were recovered for 30 min in a box flushed with 40% oxygen and then placed in their home cage.

Testing of cognitive function was performed in a 12-arm radial maze (RAM) as previously described (3–5). This RAM tests spatial working memory, assesses the integrity of the frontal cortex, entorhinal cortex and hippocampus (6,7) and can detect subtle differences in learning caused by sedatives and anesthetics (3–5,8).

To ensure motivated performance, rats were food-restricted to 85% of free-feeding body weight starting 11 days after anesthesia but had free access to water in the home cage. Rats were adapted to the maze for 11 days after anesthesia but had free access to water in the home cage. Rats were adapted to the maze for 10 min/day during days 11–13 after anesthesia, during which the maze was randomly scattered with food rewards and the rat was allowed to freely explore the maze. Formal testing began 14 days after anesthesia and consisted of a daily 15-min session in which the rat was placed on the central platform of the maze and all arms were baited. The rat was allowed to choose arms in any order until all 12 arms were visited or 15 min elapsed. A correct choice was defined as one in which the rat entered and proceeded more than 80% down a baited arm not previously explored. An error was scored when the rat entered and proceeded more than 80% down an arm it has previously visited or failed to enter the arm in 15 min. Time to complete the maze, number of correct choices before first error, and error rate were recorded.

Measures of performance from the RAM (time to complete the maze, number of correct choices to first error, and error rate) were analyzed with repeated-measures analysis of variance, with anesthesia group as a between-subject factor and day of testing as the within-subject factor. Statistical analysis of MAP and oxygen saturation was performed using one-way analysis of variance followed by Dunnett's test for multiple comparisons.

Results

Anesthesia and mechanical ventilation were physiologically well tolerated in both groups of anesthetized rats. MAP was similar in rats anesthetized with ISO and those anesthetized with ISO + N₂O and remained within the physiologic range (104 ± 2 mm Hg versus 106 ± 1 mm Hg, respectively; P > 0.05). In addition, there were no differences in oxygen saturation among rats anesthetized with ISO and ISO + N₂O (98.1% ± 0.3% versus 98.5% ± 0.3%, respectively; P > 0.05).

In two of the three measures of performance there were no significant differences between the controls and the two anesthesia groups. However, there was a significant effect of test day for each measure, indicating that learning took place across the 14 days of testing. For time to complete the maze (Fig. 1), the main effect of day was significant (P < 0.0005) but the main effect of group was not (P > 0.05) nor were there any group by day interactions (P > 0.05). For number of correct choices to first error (Fig. 2), the main effect of day was significant (P < 0.0005) but the effect of group was not (P > 0.05), nor were there any group by day interactions (P > 0.05). In terms of total number of errors (Fig. 3), there was a significant effect of day (P < 0.0005) and although there was no main effect of group (P > 0.05) the group by day interaction was significant (P < 0.05). This interaction reflects a difference in performance across days between the control group and the ISO + N₂O group. The origin of this interaction is superior performance in rats anesthetized with ISO + N₂O during later days of testing. When we compared control and ISO + N₂O groups only on the first week (days 1–7) of testing, there was no significant difference (P > 0.05) but a group difference was present during the second week (P < 0.05). Hence, general anesthesia with ISO + N₂O does not impair and may enhance acquisition of a spatial memory task administered 2 wk after general anesthesia in 6-mo old Fischer 344 rats.

Discussion

This primary finding of this study is that there is no impairment of spatial memory acquisition 2 or more weeks after general anesthesia with 1.2% ISO/70% N₂O or 1.8% ISO in adult rats. In fact, anesthesia with ISO + N₂O, but not a MAC equivalent concentration of ISO, enhanced performance on the spatial memory task. Taken together with our previous studies showing impairment in adult rats on the same task 2 days after ISO + N₂O anesthesia and in aged rats 2 weeks after anesthesia (4,5), these data support the idea that the persistent memory/learning effects of these anesthetics are age-dependent. Thus, spatial memory performance returns to normal sooner in adult rats than aged rats after general anesthesia with ISO + N₂O.

This is not the first study to demonstrate improved memory performance in rodents after anesthesia. In a previous study, in which adult rats partially learned...
the RAM task before receiving anesthesia, we found that ISO/H11001 N2O decreased their subsequent error rate (3). Similarly, the volatile anesthetics halothane, enflurane, and isoflurane have been shown to enhance memory in young adult mice (9). In those studies, over 4 consecutive days mice received 1 hour of anesthesia beginning immediately after RAM training, with the result that error rate was reduced 60%–70% (10). The mechanisms responsible for this improved performance are not known but it is interesting that memory consolidation is enhanced when natural sleep occurs soon after a learning task (11). Such improved memory performance after general anesthesia has not been reported in human surgical patients, however, perhaps because the memory improvement is subtle and difficult to detect in the ill postsurgical patient. Nevertheless, there is evidence from volunteers that visual memory for images of high emotional valence is enhanced at subanesthetic concentrations of thiopental and, possibly, propofol, but the clinical relevance of these results is unclear (12). It appears, therefore, that general anesthetics may have differential effects on different types of learning and may facilitate learning under some circumstances.

One interesting feature of the improved memory task performance in our study is that it occurred only in the group that received N2O. This is unlikely to be explained by differences in systemic physiology or depth of anesthesia, as MAP and oxygen saturation were similar and within the physiologic range in both anesthesia groups and the delivered MAC concentrations were equivalent. N2O and ISO have different receptor actions such that N2O is primarily an N-methyl-d-aspartate (NMDA) receptor antagonist with weak agonist activity at gamma-amino butyric acid (GABA) and cholinergic receptors, whereas ISO is primarily a positive GABA receptor modulator with weak NMDA receptor antagonist properties. This leads to the reasonable assumption that there was greater NMDA receptor antagonism and less GABA modulation in the ISO + N2O group than in the ISO group. Memory improvement in the former group is therefore paradoxical because activation of NMDA-glutamate receptors is required for long-term memory with weak agonist activity at gamma-amino butyric acid (GABA) and cholinergic receptors, whereas ISO is primarily a positive GABA receptor modulator with weak NMDA receptor antagonist properties. This leads to the reasonable assumption that there was greater NMDA receptor antagonism and less GABA modulation in the ISO + N2O group than in the ISO group. Memory improvement in the former group is therefore paradoxical because activation of NMDA-glutamate receptors is required for long-term memory

**Figure 1.** Average time to complete the maze. Rats (n = 10 per group) were anesthetized with 1.2% isoflurane (ISO)-70% nitrous oxide (N2O)-30% oxygen (ISO + N2O), 1.8% ISO-30% oxygen (ISO), or 30% oxygen alone (control) and tested for 14 days beginning 2 wk after anesthesia on a 12-arm radial maze. The main effect of testing day was significant (P < 0.0005) but the main effect of group was not (P > 0.05) nor were there any group by day interactions (P > 0.05). Data were analyzed with repeated-measures analysis of variance, with anesthesia group as a between subject factor and day of testing as the within-subject factor. Data points represent the mean ± SEM.

**Figure 2.** Average number of correct choices before first error. Rats (n = 10 per group) were anesthetized with 1.2% isoflurane (ISO)-70% nitrous oxide (N2O)-30% oxygen (ISO + N2O), 1.8% ISO-30% oxygen (ISO), or 30% oxygen alone (control) and tested for 14 days beginning 2 wk after anesthesia on a 12-arm radial maze. The main effect of testing day was significant (P < 0.0005) but the main effect of group was not (P > 0.05) and nor were there any group by day interactions (P > 0.05). Data were analyzed with repeated-measures analysis of variance, with anesthesia group as a between subject factor and day of testing as the within-subject factor. Data points represent the mean ± SEM.

**Figure 3.** Average number of errors. Rats (n = 10 per group) were anesthetized with 1.2% isoflurane (ISO)-70% nitrous oxide (N2O)-30% oxygen (ISO + N2O), 1.8% ISO-30% oxygen (ISO), or 30% oxygen alone (control) and tested for 14 days beginning 2 wk after anesthesia on a 12-arm radial maze. There was a main effect of testing day (P < 0.0005) and although there was no main effect of group (P > 0.05), the group by day interaction was significant and reflected superior performance in rats anesthetized with ISO + N2O during the later days of testing. Data were analyzed with repeated-measures analysis of variance, with anesthesia group as a between subject factor and day of testing as the within-subject factor. Data points represent the mean ± SEM.
formation (13,14). However, the effect of NMDA receptor antagonists on the capacity of the adult brain to learn days or weeks later have not been studied extensively and there is evidence for both impaired acquisition (15) as well as better retention of certain memory tasks (16). In addition, in cultured hippocampal slices, prolonged NMDA receptor blockade results in axonal sprouting and more miniature excitatory postsynaptic potentials (17), which are morphological and neurophysiological features of memory-dependent synaptic plasticity. Thus, there is limited behavioral and cellular evidence for a potential facilitating effect of certain NMDA antagonists on memory-dependent synaptic plasticity, but whether these explain the enhanced learning we observed on a hippocampus-dependent spatial memory task after ISO + N₂O anesthesia is unknown.

This study is limited in a number of ways. First, although the anesthetized animals were tracheally intubated and mechanically ventilated, the controls were not. It is unlikely that the improvement in maze performance in the ISO + N₂O anesthetized rats was attributable to tracheal intubation and mechanical ventilation, as the ISO group was treated identically but showed no behavioral improvement. Second, the RAM task depends on hunger-induced motivation and the ability to see extra-maze visual cues. However, all rats included in the study learned the maze, had similar weights for the 11 days after anesthesia before RAM testing, and consumed food pellets when returned to their cage, indicating that they were able to see visual cues and were motivated by hunger. Finally, the RAM tests spatial memory, and it is possible that other cognitive domains may be more or less sensitive to anesthesia-induced changes.

It is difficult to understand how general anesthesia ablates memory during the time it is administered, impairs it 48 hours later irrespective of age, and yet subsequently enhances long-term memory performance in young animals and impairs it in the old. However, memory itself is a complicated and poorly understood phenomenon and our understanding of how general anesthetics influence it is also poor. Anesthetics have known effects on many of neurochemical events associated with memory-dependent synaptic plasticity (18), but it is impossible to speculate how they may influence memory acquisition over the longer term.

In summary, our results show that in contrast to the persistent impairment observed in aged rats (5), MAC equivalent dosages of ISO + N₂O and ISO alone do not impair spatial memory acquisition 2 weeks later in adult rats. In fact, previous anesthesia with ISO + N₂O actually improves RAM performance 2 weeks later. Together with previous work, these results indicate that postanesthetic memory impairment is age-dependent such that adult rats recover sooner than aged rats. Because improved task performance 2 weeks after anesthesia can not be explained by the pharmacokinetics of the drugs involved, these data suggest that persistent anesthesia-induced changes occur in neural structures and/or biochemical cascades mediating memory. Such lasting cognitive changes provide a basis for examining further the long-term neurobiological effects of anesthesia on learning and memory.

References